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NOTE

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A clinical case of presumed cerebellar medulloblastoma in a Japanese Black calf with increased neuron-specific enolase in cerebrospinal fluid

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ABSTRACT. A 2-day-old Japanese Black male calf that presented with opisthotonos with spastic extension of all four limbs and nystagmus was presented. Evaluation of cranial neurology revealed a horizontal slow nystagmus and absence of menace response in the left eye. Necropsy revealed a mass located between the posterior margin of the cerebrum and anterior margin of the cerebellum, and continuously with the cerebellar lesion. The brainstem was severely compressed by those lesions. Original structures of the cerebellum were mostly replaced by grayish-white and brownish tissues. Those lesions were diagnosed as presumed cerebellar medulloblastoma by histopathological and immunohistochemical examination. As neuron-specific enolase in the cerebrospinal fluid which is a biomarker for neuronal damage was increased compared with healthy calves.

KEY WORDS: calf, cerebellar medulloblastoma, Japanese Black, neuron-specific enolase

Cerebellar medulloblastoma is a type of malignant tumor derived from the germinal layer that can develop shortly after birth or in young animals [13]. The cerebellar vermis is the most common site of tumor formation. The tumor is highly invasive and spreads to the fourth ventricle and the entire central nervous system [13]. Although cerebellar medulloblastoma is a relatively rare neoplasia in cattle, several cases have been reported in calves or heifers younger than age 12 months [1–3, 5, 6, 8, 12–14, 16, 18]. However, no such reports have been published on Japanese Black, a beef cattle of Japanese origin. Here we report a case of presumed cerebellar medulloblastoma in a newborn Japanese Black calf with opisthotonos since birth. The ability of neuron-specific enolase (NSE) in cerebrospinal fluid (CSF) to serve as a potential biomarker for neuronal damage was also evaluated.

A 2-day-old Japanese Black male calf presented to a local veterinarian with a chief complaint of astasia since birth. Since the calf presented with opisthotonos with spastic extension of all four limbs and nystagmus, the veterinarian suspected a congenital disorder, meningitis, or encephalitis. Although the body temperature of the calf was not high (38.6°C), enrofloxacin and dexamethasone were administered due to the possibility of bacterial infection until Day 4, but clinical signs did not improve. The calf was transferred to the Veterinary Teaching Hospital of Obihiro University of Agriculture and Veterinary Medicine on Day 7. The calf presented with a tremor with lateral recumbency and opisthotonos (Fig. 1) but showed clear consciousness and drunk milk with assistance. Physical examination revealed normal respiration and a strong inhalation reflex with a normal temperature of 39.0°C. Spinal cord reflex could not be evaluated due to spastic extension of all four limbs. Evaluation of cranial neurology revealed slow horizontal nystagmus and absence of menace response in the left eye. White blood cell count was within the normal range (5,400/μl). Serum total protein, albumin, and A/G ratio were 5.1 g/dl, 2.5 g/dl, and 1.04, respectively, all within the normal range. Serum protein electrophoresis fractionation suggested the lack of inflammation. Bovine viral diarrhea virus was not detected by RT-PCR.

CSF was collected by cerebellomedullary cisternal puncture. Increased CSF pressure was noted during collection, although

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Fig. 1. The calf presented with lateral recumbency and opisthotonos (Day 7).

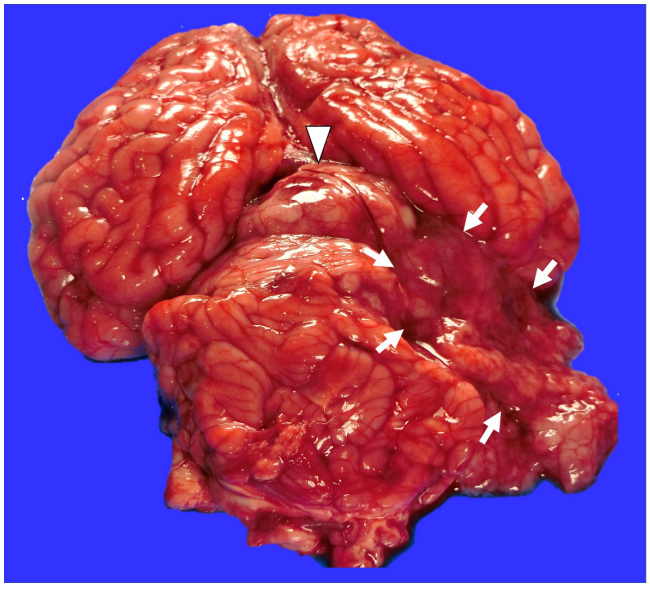

Fig. 2. The cerebellum was increased in volume, and the right dorsal portion of cerebellar vermis was fragile and flattened and lost its original shape (arrows). A tumor 2 cm in diameter was detected between the posterior margin of the cerebrum and anterior margin of the cerebellum (arrowhead).

the fluid was colorless and transparent. Number of cells, specific gravity, and protein concentration of CSF were <100/μl, 1.010, and 210 mg/d*l*, respectively. Protein concentration was slightly increased relative to the reference range (<30 mg/d*l*) reported in a previous study [1]. NSE in CSF was quantified using an ELISA kit for NSE (Immunospec,West Hills, CA, USA). The concentration of NSE in CSF was 9.6 *n*g/m*l*, which was higher than the mean NSE value in CSF from 9 clinically healthy calves aged 8–30 days (mean ± standard deviation, 1.68 ± 0.63 *n*g/m*l*).

The calf died on Day 8, and necropsy was performed on the same day. Protrusion of the cerebellum from the foramen magnum suggested cerebellar hernia. The cerebellum was also increased in volume, and the right dorsal portion of the cerebellar vermis had lost its original shape with softening and flattening of the parenchyma (Fig. 2). The structure of cerebellar folia had also disappeared. A mass 2 cm in diameter was detected between the posterior margin of the cerebrum and anterior margin of the cerebellum and continuously with the cerebellar lesion (Fig. 2). The mass was composed of grayish-white and brownish tissues. On the cut surface of the cerebellum, original structures of the cerebellum, especially of the vermis and the right hemisphere, were mostly replaced by the same tissues of the neoplastic mass. In addition, the brainstem was severely compressed by those tissues. Tumor growth was restricted to the cerebellum, and no tumor cells were found in the cerebrum and brainstem. In the relatively preserved cerebellar folium, tumor cell proliferation was also seen in the area of external granular layer (Fig. 3). Tumor cells with a circular to elliptical nucleus proliferated in a solid manner while forming Homer-Wright rosettes and ependymal rosettes with concentric eosinophilic rings in the centers (Fig. 4). The tumor cells had round to elongate nuclei without apparent cytoplasm (Fig. 4). Mitotic figures were 1 to 2/HPF. Foci of necrosis were observed in the neoplastic tissues. Necrotic areas were also seen in the involved cerebellar tissues with foamy macrophage infiltration. Immunohistochemically, tumor cells were stained positive with a neuronal marker, anti-microtubules associated protein (MAP) 2 mouse monoclonal antibody (Kamiya Biomedical Co., Seattle, WA, USA), and stained negative with anti-glial fibrillary acidic protein mouse monoclonal antibody (GFAP: DAKO, Glostrup, Denmark) (Fig. 5). The tumor was diagnosed as a cerebellar primitive neuroectodermal tumor (PNET), refereed as presumed cerebellar medulloblastoma, on the basis of the location and histopathological findings. However, ependymomas should also be considered, as they are known to form in the cerebellum of young cows [17]. More diagnostic approach is required to confirm the pathological diagnosis of cerebellar meduloblastoma.

At the initial visit to the local veterinarian, congenital cerebellar malformation, meningitis, and encephalitis were differential diagnoses based on the calf's clinical symptoms. Antibiotics and steroids were administered to control a potential bacterial infection, but meningitis and encephalitis were ruled out since clinical signs did not improve after 4 days of treatment. This was also supported by the lack of inflammation as assessed by blood cell counts, serum protein analysis, and CSF analysis. Thus, congenital anomalies which might cause opisthotonos, including hydrocephalus, cerebellar hypoplasia, and fetal brain tumor, were suspected. When CSF was collected, increased pressure was noted, suggesting the possibility of hydrocephalus or volume-increasing diseases such as a brain tumor. However, a definitive antemortem diagnosis could not be made. The present case was diagnosed postmortem as presumed cerebellar medulloblastoma with cerebellar hernia by pathological examination. This diagnosis explained most of the findings, including lateral recumbency, opisthotonos, and increased intracranial pressure. Although the tumor

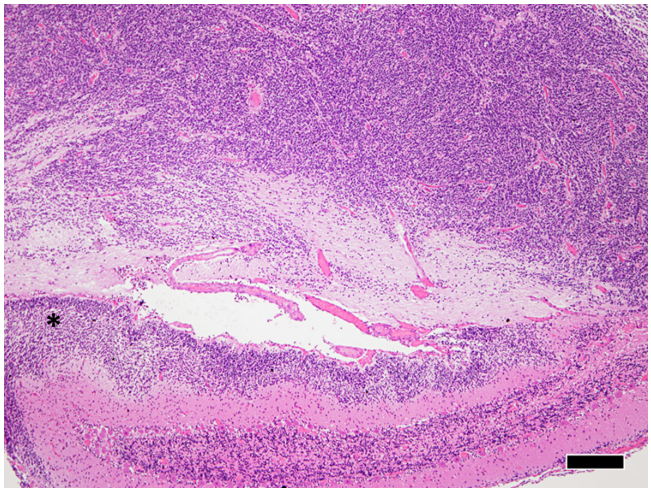

Fig. 3. In the relatively preserved cerebellar folium, tumor cell proliferation is also seen in the area of external granular layer (asterisk). Hematoxylin and Eosin stain (HE). Bar=200 μm.

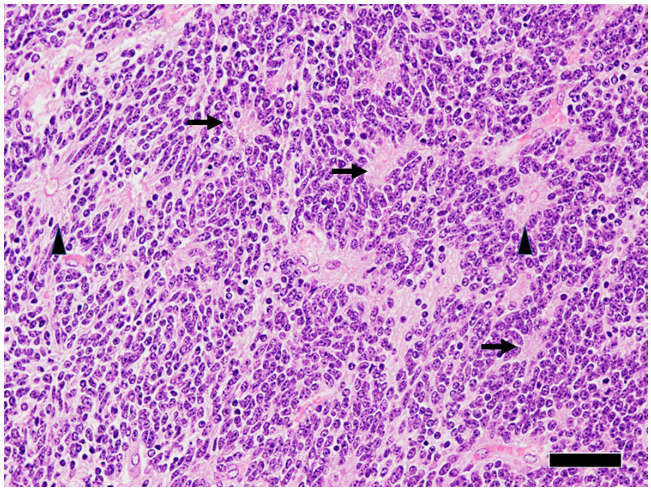

Fig. 4. Tumor cells with a circular to elliptical nucleus proliferated in a solid manner while forming Homer-Wright rosettes (arrows) and ependymal rosettes with concentric eosinophilic rings in the centers (arrowheads). Hematoxylin and Eosin stain (HE). Bar=50 μm.

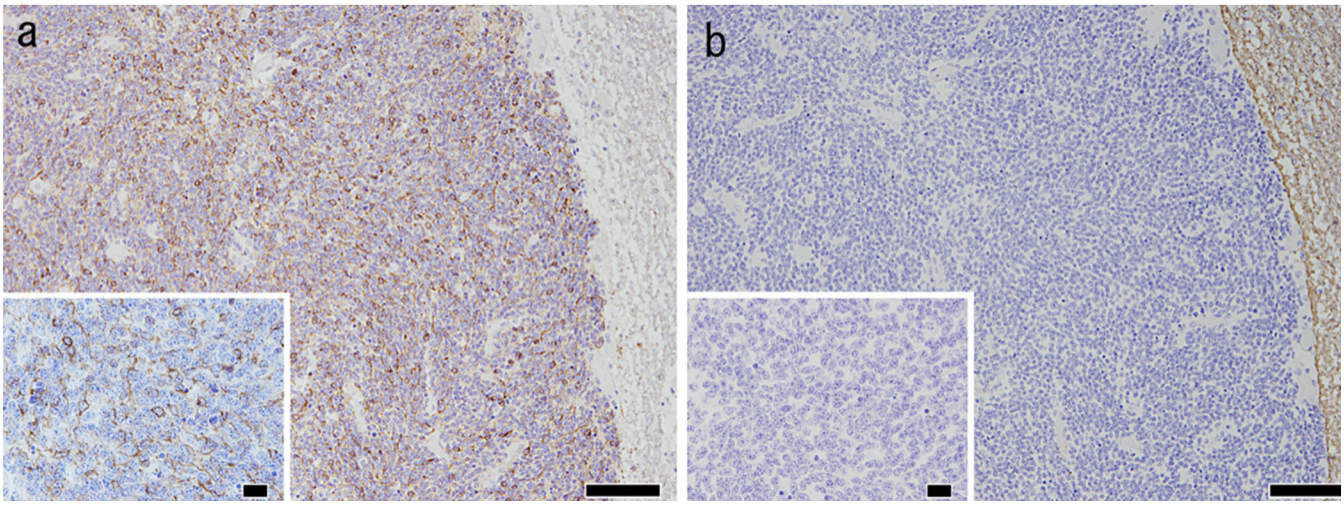

Fig. 5. Tumor cells are stained positive with microtubules associated protein-2 (a) and negative with glial fibrillary acidic protein (b). Immunohistochemistry. Hematoxylin counterstain. Bars=100 μm.

was located mainly on the right dorsal side of the cerebellum, the tumor aggressively invaded the cerebellum and pressed on the brain stem. Brainstem symptoms such as horizontal nystagmus and disappearance of menace reaction were observed on the left side, possibly reflecting impairment of cranial nerves VII and VIII due to compression of the left brainstem by the tumor [10].

Several case reports of cerebellar medulloblastoma have been reported in cattle breeds such as Holstein Friesian, Aberdeen Angus, Hereford, cross breed of Aberdeen Angus and Hereford, Australian Shorthorn, Flemish, and Brazilian Girolands [1–3, 5, 6, 8, 12–14, 16, 18]. The present case report is the first to describe a clinical case of presumed cerebellar medulloblastoma in a Japanese Black calf, the major beef cattle breed in Japan. Although very rare, cerebellar medulloblastoma should be included in the differential diagnosis for Japanese Black calves when symptoms are associated with cerebellar and/or brainstem abnormalities, including lateral recumbency, opisthotonos, and loss of menace response, symptoms most commonly reported in previous studies [1–3, 5, 6, 8, 12–14, 16, 18].

In addition to detecting tumor cells in CSF, computed tomography (CT)/magnetic resonance imaging (MRI) can assist in antemortem diagnosis of medulloblastoma in clinical settings [4, 7]. Although a recent study reported the use of these methods in diagnosing a heifer with cerebellar medulloblastoma [5], in reality diagnostic imaging is extremely difficult in large animals, especially in cattle. In the present case, we evaluated the concentration of NSE in CSF and found it to be higher compared to that observed in normal calves. NSE is a glycolytic pathway isozyme which is present predominantly in the cytoplasm of neurons [11]. NSE in CSF has been reported to be a useful biomarker for neuronal damage in human medicine [15]. In bovine medicine,

only a few studies have assessed neuron biomarkers such as S100B [9]. Increased NSE in CSF found in the present case might be originated from neuronal damage by cerebellar medulloblastoma or necrosis of the cerebellum. Our present findings suggest that NSE in CSF may serve as a biomarker of neuronal damage in cattle as well and may allow for predicting prognosis. Future studies that assess the utility of NSE as a prognostic biomarker in bovine medicine are warranted.

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